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The Cell Wall in Ripening Avocados

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Introduction

Abnormalities in ripening lead, in some years, to substantial losses of fruit. For example, in tomato, there is a physiological disorder known as "blotchy" fruit which has been related to alterations in the cell wall metabolism (7). Some areas within the fruit fail to soften normally resulting in hard spots in the otherwise ripe fruit. This reduction in quality of the fruit often results in rejection from the market. A similar disorder has been reported in the avocado fruit. Oppenheimer (11) found that low yield (related to temperatures or individual fruiting habits of the trees), delay in harvest, large size of fruit, and non-adhering seed-coats were factors which seemed to be associated with a high percentage of abnormal fruit. In order to reduce these abnormalities we must learn more about the cell wall changes taking place during the normal softening process.

The cell wall is an additional layer that plants have outside the membrane that delimits each cell. Among other things, the wall gives the plant cell rigidity, and functions in support and as a protection against attack by pathogens (6). In general, cell walls in plants are made up primarily of four polymeric structures that are identified by their solubility: pectins that are soluble in water and dilute alkali, hemicelluloses which can be solubilized with concentrated alkali, cellulose and lignin that are insoluble in alkali (10). Pectins are made up of long chains of galacturonic acid, arabinose, and galactose, while hemicelluloses are polymers of xylose, arabinose, rhamnose, and other sugars. On the other hand, cellulose is made up of long chains of glucose molecules in ß-1,4 linkages (15). Lignin is a very complex substance not commonly found in fruits. Awad and Young (4) found that polygalacturonase, the enzyme which hydrolyzes partially methylated pectin to galacturonic acid, is very active during the latter part of the ripening process. The activity of another enzyme which hydrolyzes carboxymethylcellulose (cellulase) shows slight activity in freshly-picked hard avocado fruit, increases markedly just as softening progresses, and attains the highest activity ever observed for a cellulase from a plant source in the over-ripe stage. However, the importance of this enzyme in relation to softening of the avocado fruit has not bean clearly determined.

It was the purpose of these studies to determine what polysaccharides of the cell wall are hydrolyzed during ripening, and to learn more about the role of cellulase in the

softening of the avocado fruit.

Materials and Methods

Mature "Hass" avocado fruit at six stages of ripeness were used in this study: (I) freshly picked fruit, (II) an early pre-softening stage, (III) a late pre-softening stage, (IV) soft, ripe stage, (V) two days, and (VI) three days past the best ripe stage. Cell walls were extracted according to the method of Talmadge et al. (14). Uronic acid concentrations (pectins) were determined by the method of Ahmed and Labavitch (1). The cellulose content of the cell walls was determined as described by Ahmed and Labavitch (2). The total neutral sugars (from pectins and hemicelluloses) were assayed by the Nelson method (9).

The enzyme cellulase was extracted from soft avocado fruit according to the method of Awad and Young (4) and later purified as described by Awad and Lewis (3). The purified enzyme was assayed viscometrically using carboxymethylcellulose as the substrate. The enzyme's activity was also assayed colorimetrically using various commercial polysaccharides.

Table I. Yield of Cell Wall from Avocado Fruit at Various Stages of Ripeness.

Stage of Ripeness	Yield (mg cell wall / g mesocarp dry weight)	Relative Loss
I	137	100
II	132	96
III	135	98
IV	105	77
V	79	58
VI	65	47

Stages I through VI relate to stages of ripeness as described under Materials and Methods.

Each figure under "yield" is the average of 7 different extractions.

Results and Discussion

The yield of cell wall per unit of mesocarp dry weight did not change during the first three stages of ripeness considered, but decreased sharply afterwards (stages IV, V, and VI). As can be seen in Table I, the overall loss was about 53%. The solubilization of the cell wall as ripening proceeded was reflected in a marked loss of the main cell wall components (Table II). A slight decrease in uronic acids and neutral sugars could be observed in the first stages considered, and after stage III the decrease in content of these components became much more evident, as was also the case of cellulose which did not show any changes initially. About 43% of the initial cellulose content was lost in the later stages of ripening. On the other hand, about 70% of the initial content of uronic acids and about 58% of the initial reducing sugars were lost during the whole ripening process. These results indicate that in addition to pectin changes, which have been well studied by several authors (5, 8, 13), changes in neutral sugars and cellulose are also taking place during ripening of the avocado which may influence the textural changes associated with fruit softening. This area invites further research.

Although cellulase activity increases very early during ripening of the fruit (4), the results of the present study show that cellulose is lost from the cell wall only after solubilization of other cell wall components (uronic acids and reducing sugars) had been initiated, which led us to believe that cellulase was probably acting more like a hemicellulase hydrolyzing other polysaccharides.

Table II. Changes in the Content of Various Components of the Avocado Cell Walls.

Component.	Stages of Ripeness					
	I	II	III	IV	V	VI
	mg/g mesocarp dry weight					
Uronic Acids	49.3	48.6	47.0	28.0	19.2	15.1
Reducing Sugars	47.8	44.9	43.3	35.6	23.1	20.1
Cellulose	28.0	27.8	28.3	23.4	18.7	16.1

Stages I through VI relate to stages of ripeness as described under Materials and Methods.

The results for the different components are based on triplicate samples.

In order to learn more about the action of the avocado eellulase, its capacity to hydrolyze polysaccharide substrates with various structural characteristics was

measured. The purified avocado cellulase was very effective in hydrolyzing carboxymethylcellulose, which is a very desirable substrate for the assay of cellulase. The enzyme had an activity of about 8000 units/g fresh weight when assayed viscometrically. However, when the colorimetric method was used, a long time of incubation (more than 2 hours) was required to detect a measurable amount of reducing sugars, which suggests that the purified enzyme is an endo cellulase (Table III). The enzyme was also very effective in hydrolyzing Sigmacell (highly purified cellulose fibers), especially when this substrate was treated with 85% phosphoric acid (Table III). The purpose of this treatment was to produce a highly reactive cellulose which could give a good indication of the enzyme's activity within a relatively short time. On the other hand, the cellulase showed a minimal activity toward the commercial polysaccharides laminarin, xylan, and arabino-galactan (Table III), thus corroborating that the enzyme was a specific cellulase.

It seems that, although the enzyme does not seem to contribute to early loss of firmness in the fruit, it is probably responsible for cellulose hydrolysis late in the ripening process. But the fact that cellulase has been found even in mature hard fruit (4, 12) indicates that this enzyme probably has another function that has not been detected and should be investigated further.

Table III. Activity of Purified Avocado Cellulase on Different Substrates.

Substrate	Activity
Laminarin	0.068
Xylan	0.051
Arabino-galactan	0.060
Carboxymethylcellulose	1.700
Sigmacell (untreated)	0.302
Sigmacell (treated with acid)	1.312

Activity is given in μ moles reducing groups/g fresh weight/7 hours.

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